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UTILIZATION OF NOVEL SUSTAINABLE FEED MATERIALS IN AQUAFEED TOWARDS THE FORTIFICATION OF GILTHEAD SEABREAM (*Sparus aurata*)

Pedro Campelos Ribeiro

Dissertação de Mestrado apresentada à
Faculdade de Ciências da Universidade do Porto
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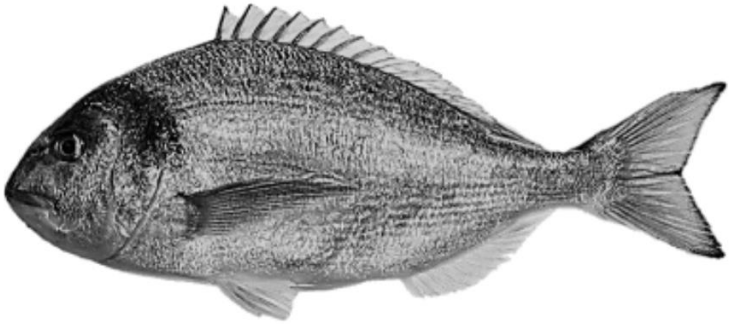

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Departamento de Biologia
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Orientador
Professora Doutora Luísa Maria Pinheiro Valente

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O Presidente do Júri,

Porto, / /

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Abstract

Aquaculture is a thriving and important food production sector worldwide. Increase demand for food fish has driven aquaculture production towards tremendous expansion in terms of volume and value. Growing production from aquaculture results in an increase seek for fish feed and consequently in a constant need for fish meal and fish oil as feed sources. However, reduced availability and increased costs of those resources compromise aquaculture development and consequent fish and seafood supply for human consumption. Aquaculture industry has been challenged to find healthy and sustainable solutions to meet consumers eating habits increasingly moving towards healthy and natural food items. The replacement of the traditional fish meal and fish oil in aquafeeds with sustainable solutions like algae has instigated interest due to their high nutritional value and richness in bioactive compounds. In this context, natural feed ingredients like macroalgae, microalgae and yeast biomass were exploited as an alternative to fish meal and fish oil, as well as vehicles for bio-fortification of fish in iodine, selenium and n-3 polyunsaturated fatty acids. In the present study, gilthead seabream were fed for ten weeks with four diets: a commercial-based diet with fish meal, FM, and fish oil, FO (FMFO) and three experimental diets with a 33% replacement of FM by a microalgae biomass, AB (*Chlorella, Tetraselmis* and *Schizochytrium*) (AB diets). A further replacement of 20% FO was tested in ABVO diet. All AB rich diets were supplemented with both *Laminaria* and selenium-yeast (Se-yeast): diets $ABVO_{I8+Se1}$ and $ABFO_{I8+Se1}$ provided equivalent levels of iodine (I) (8 $mg.kg^{-1}$) and selenium (Se) (1 $mg.kg^{-1}$), whereas an extreme diet $ABFO_{I15+Se1.4}$ was further enriched with I (15 $mg.kg^{-1}$) and Se (1.4 $mg.kg^{-1}$). At the end of the trial, growth performance, whole body composition and the expression of genes involved in fish metabolism were determined in liver and muscle. Final body weight of fish fed the bio-fortified diets was similar to those fed the FMFO diet, with the exception of the $ABFO_{I15+Se1.4}$ that resulted in a significantly lower body weight. This diet also showed a significantly lower protein efficiency ratio (PER) than all other diets. The specific growth rate (SGR) and feed conversion ratio (FCR) remained similar among dietary treatments. The expression of genes related to fish metabolism varied both in liver and muscle with the dietary treatments. In liver, the expression of *cpt1a* gene, which provides instruction for the enzyme carnitine palmitoyltransferase, was downregulated in fish fed $ABVO_{I8+Se1}$ diet. In muscle, the higher iodine content in the $ABFO_{I15+Se1.4}$ diet, as a result of *Laminaria* incorporation, was associated with an

upregulation of the *gpx1a* gene, probably due to an increased anti-oxidative metabolism. The present results indicate that the concomitant replacement of 33% FM and 20% FO by a blend of microalgae biomass (*Chlorella, Tetraselmis* and *Schizochytrium)* can be achieved without any significant impact on specific growth rate or whole-body composition of gilthead seabream. However, the highest dietary levels of both Se and I resulted in reduction of fish final size. In conclusion, the use of algae biomass as a sustainable alternative to fish meal and fish oil in gilthead seabream in diets is possible but requires a cautious selection of ingredients at adequate level to avoid growth impairment.

Keywords: Gilthead seabream; Fish nutrition; Algae; Gene expression; Metabolism;

Resumo

Aquacultura é um importante sector de produção alimentar em expansão a nível mundial. O aumento da procura de peixe levou a um enorme aumento da produção em aquacultura em termos de volume e valor comercial. O crescimento da produção em aquacultura resulta no aumento da procura por rações para peixe e consequentemente na constante necessidade em farinha e óleo de peixe como fontes de alimento. No entanto, a reduzida disponibilidade e aumento no custo destes recursos compromete o desenvolvimento da aquacultura e o fornecimento de peixe e alimentos provenientes do mar para consumo humano. A indústria da aquacultura foi desafiada a encontrar soluções saudáveis e sustentáveis para ir ao encontro dos novos hábitos alimentares dos consumidores cada vez mais voltados para alimentos saudáveis e naturais. A substituição da tradicional farinha e óleo de peixe, usados em rações, por soluções sustentáveis, como as algas, despertou interesse devido ao seu alto valor nutricional e riqueza em compostos bioativos. Nesse sentido, ingredientes naturais para alimentação animal, como macroalgas, microalgas e leveduras, foram utilizados como alternativa à farinha e óleo de peixe, mas também como veículos para a biofortificação de peixes em iodo, selénio e ácidos gordos polinsaturados. No presente estudo, douradas foram alimentadas durante 10 semanas com quatro dietas: uma dieta comercial com farinha de peixe, FM, e óleo de peixe, FO (FMFO) e três dietas experimentais com substituição de 33% de FM por uma biomassa de microalgas, AB (*Chlorella*, *Tetraselmis* e *Schizochytrium*) (dietas AB). Uma substituição adicional de 20%

de FO foi testada na dieta ABVO. Todas as dietas ricas em AB foram suplementadas com *Laminaria* e levedura de selénio (Se-yeast): as dietas $ABVO_{I8+Se1}$ e $ABFO_{I8+Se1}$ forneceram níveis equivalentes de iodo (I) (8 $mg.kg^{-1}$) e selénio (Se) (1 $mg.kg^{-1}$), enquanto uma dieta exagerada $ABFO_{I15+Se1.4}$ foi enriquecida com I (15 $mg.kg^{-1}$) e Se (1.4 $mg.kg^{-1}$). No final do estudo, foram avaliados o desempenho no crescimento, a composição corporal e a expressão de genes envolvidos no metabolismo dos peixes. O peso corporal final dos peixes alimentados com as dietas biofortificadas foi semelhante ao da dieta FMFO, com exceção do $ABFO_{I15+Se1.4}$, que resultou num peso corporal significativamente menor. Essa dieta também apresentou uma taxa de eficiência proteica (PER) significativamente menor do que todas as outras dietas. A taxa de crescimento específico (SGR) e a taxa de conversão alimentar (FCR) permaneceram semelhantes entre todas as dietas. No fígado, a expressão do gene *cpt1a*, que fornece instruções para a enzima carnitina palmitoiltransferase, foi regulada negativamente em peixes alimentados com a dieta $ABVO_{I8+Se1}$. No músculo, o maior teor de iodo na dieta $ABFO_{I15+Se1.4}$, como resultado da incorporação de *Laminaria*, foi associado a uma regulação positiva do gene *gpx1a*, provavelmente devido a um aumento do metabolismo anti-oxidativo. Os resultados apresentados indicam que a substituição simultânea de 33% FM e 20% FO por uma mistura de microalgas (*Chlorella*, *Tetraselmis* e *Schizochytrium*) pode ser alcançada sem qualquer impacto significativo na taxa de crescimento específico ou na composição corporal de douradas. Em conclusão, o uso de biomassa de algas como alternativa sustentável à farinha e óleo de peixe em dietas de douradas é possível, mas requer uma seleção cautelosa de ingredientes em níveis adequados por forma a evitar disparidades no crescimento.

Palavras-chave: Dourada; Nutrição em peixes; Algas; Expressão de genes; Metabolismo;

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Abbreviations

FM – Fish meal
FO – Fish oil
Se-yeast – Selenium yeast
Se – Selenium
I – Iodine
EPA – Eicosapentanoic acid
DHA – Docosahexaenoic acid
PER – Protein efficiency ratio
DGI – Daily growth index
SGR – Specific growth ratio
FCR – Feed conversion ratio
HSI – Hepatosomatic índex
KI – Condition index
EAA – Essential amino acids
N – Nitrogen
HUFA – Highly unsaturated fatty acids
PUFA – Polyunsaturated fatty acids
IDD – Iodine deficiency disorders
T4 – Thyroxine hormone
T3 – Triiodothyronine hormone
Se-Met – Selenomethionine
FL – Fork length
TL – Total length
RNA – Ribonucleic acid
DNA – Deoxyribonucleic acid
cDNA – Complementary DNA
PCR – Polymerase chain reaction
SFA – Saturated fatty acids
MUFA – Monounsaturated fatty acids
ALA – α-Linolenic acid
LA – Linoleic acid

Introduction

Aquaculture today

Aquaculture is the fastest-growing animal food production sector worldwide, with an average annual growth of 5.8 percent during the period 2000-2016 (FAO, 2018; Ottinger, Clauss, & Kuenzer, 2016). Global aquaculture production reached a total volume of about 80 million tonnes of food fish in 2016, with an estimated first sale value of USD 232 billion (FAO, 2018). In 2016 aquaculture represented about 47 percent of world fish production (Fig. 1) (FAO, 2018) and is becoming the main source of aquatic animal food in human consumption (Béné et al., 2015; Toufique & Belton, 2014). On a global scale, food fish consumption peaked at about 20.2 kg per capita in 2015 and it is expected to continue growing (FAO, 2018). The world population is projected to growth to nearly 9.7 billion people by 2050 (ONU, 2019) and an associated economic development will increase future demand for fish products. Captured fishery production relatively static since the early 1990s, depletion of wild fishery stocks, rising global populations, continuing demand for food fish and international trade has driven aquaculture impressive growth during the last decades (Ottinger et al., 2016). Therefore, aquaculture can make an important contribution in terms of fish food production (Béné et al., 2015; Natale, Hofherr, Fiore, & Virtanen, 2013; Naylor et al., 2002), balancing the stagnated production volumes from capture fisheries providing continuous growth in the supply of fish for human consumption but also reduce the pressure on natural resources (Lee & Yoo, 2014). However, the rapid growth of aquaculture has heavily relied on fish meal and fish oil as main sources of aquafeed due to their high digestibility and high nutritional value. Fish meal and fish oil provide a balanced amount of essential amino acids, fatty acids, phospholipids and minerals. Fish meal and fish oil are still mainly obtained from wild-harvested pelagic fish populations like anchovy, sand eel, sardine, capelin and herring, which stocks are fully or overexploited, as they are vulnerable to collapse because of poor management, overfishing, and climate variations. Therefore, given the limited supply of fish meal and fish oil from wild catches, the efficient use and share of these products is a major issue for the aquaculture industry (Kaushik and Troell, 2010; Tacon and Metian, 2009). Although fish meal and fish oil are still considered the most nutritious and most digestible ingredients for farmed fish feeds, fluctuations in production volume and variation in market price represent a clear decrease in their availability for

aquafeed manufacture. Based on these facts, it is expected that future demand for aquafeeds to support aquaculture growth will be driven by industry capacity to reduce the dependency on fish meal and fish oil obtained from wild fish stocks, or better yet, not depend on it at all (Delgado, Wada, Rosegrant, Meijer, & Ahmed, 2003; FAO, 2018). As a thriving and important food supply sector worldwide, aquaculture requires the search for alternative solutions as aquafeed over-reliance on fish meal and fish oil can be unsustainable and compromise marine biodiversity and human food security. Further still, aquaculture must ensure that these alternative sources do not compromise the nutritional quality, health and welfare of animals and therefore the production of a high consumer-quality final product (FAO, 2018; FAO STAT, 2013). This need has resulted in the research of new environmentally sustainable food sources in the production of aquafeeds, namely plant-based substitutes or even in the use of macroalgae and microalgae in the partial or total replacement of fish meal and fish oil. However, some plant-based alternatives have been proven to represent some disadvantages in its use, as low digestibility, deficiency in certain essential amino acids and some antinutritional factors, resulting in significant changes in the nutritional quality of the final product. Instead, algae could have significant beneficial effects and could potentially replace or reduce common feed stuff due to their content in essential amino acids, polyunsaturated fatty acids, vitamins and minerals, representing a high nutritional and balanced feed source (P. Li, Mai, Trushenski, & Wu, 2009; Turchini, Torstensen, & Ng, 2009). Fish is generally regarded as a healthy food being a rich source of essential nutrients, including proteins, lipids, vitamins and minerals. It exerts beneficial effects on several body functions improving health and well-being. Current trend on healthy eating habits by general consumers has driven consumption towards healthy, natural and functional foods. Therefore, growth and development of aquaculture industry will depend on scientific and technological advances to meet current challenges on the production of high nutritional and environmentally sustainable products.

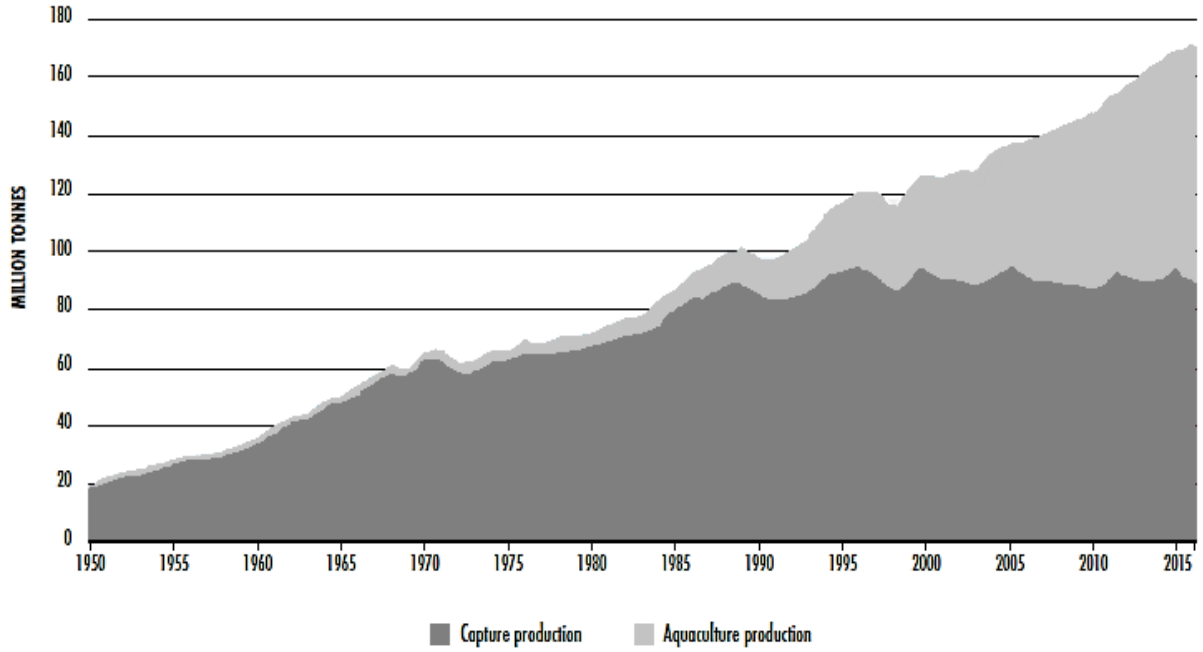

Figure 1 World capture fisheries and aquaculture production (FAO, 2018)

Gilthead seabream (*Sparus aurata*)

Gilthead seabream (*Sparus aurata*, Linnaeus, 1758) (Fig. 2) belongs to the order Perciforms, family Sparidae and genus *Sparus*. Species anatomically features an oval body shape, rather deep and compressed, and a head profile regularly curved. Mouth is located low and have small eyes. Fish body has a silver-grey colour with a large black blotch at the origin of lateral line, extending on upper margin of operculum where it is edged below by a reddish area. Dark longitudinal lines are often present on sides of body and a dark band on dorsal fin can also be identified. A pronounced black edged fork and tips of caudal fin and a distinctive golden frontal band between eyes edged by two dark areas also characterizes this species (FAO, 2017).

Figure 2 Gilthead seabream (Sparus aurata, Linnaeus, 1758)

Biology and Habitat

Anatomically, gilthead seabream possesses the mandible shorter than maxilla and each jaw has four to six canine-like teeth anteriorly located, followed posteriorly by blunter teeth which become progressively molar-like arranged in two to four rows. Dorsal fin has eleven spines and thirteen to fourteen soft rays whilst anal fin has three spines and eleven or twelve soft rays (FAO, 2017). This species is a protandrous hermaphrodite that first evolve as males and later either morphing into females or living on as males. Gilthead seabream juveniles reach sexual maturity as males during the second year of life (20-30 cm) whereas female sexual maturity develops at two to three years of life (33-40 cm), generally at the second spawning season. In captivity conditions, sex reversal is conditioned by social and hormonal factors (Russell, Carpenter, & Pollard, 2014). During the male phase, the bisexual gonad has testicular tissue, with asynchronous spermatogenesis and non-functional ovarian areas (Crosetti & Innocentiis, 2014). *S. aurata* is a multiple spawning species. Spawning season generally occurs from October to December, with sequenced spawning during the whole period. The species spawns pelagically, most probably in group-spawning aggregations in mid water (Russell et al., 2014). Ovarian development is also asynchronous, and females can lay 20 000-80 000 eggs every day during spawning period. The eggs are spherical and pelagic, with a diameter around 1 mm and a single large oil droplet. The planktonic larval stage lasts about 50 days at 17-18°C (Koven, 2002). *S. aurata*

is mainly carnivorous, feeding on zoobenthos, such as mollusks, particularly mussels which it can easily crush, decapods, annelids, crustaceans and fish, although it is accessorily herbivorous (Crosetti & Innocentiis, 2014). Gilthead seabream is a costal species, inhabiting seagrass beds, sandy bottoms, and the surf zone, commonly down to 30 m, living solitary or in small aggregations. However, adults may occur in deep waters at nearly 150 m (Crosetti & Innocentiis, 2014). This species can be found throughout the Mediterranean Sea, present along the Eastern Atlantic coasts from Great Britain to Senegal but not so common in the Black Sea (Fig. 3). *S. aurata* is an euryhaline and eurythermal species, and so the species can be found in both marine and brackish water environments such as coastal lagoons and estuarine areas, in particular during the initial stages of its life cycle (FAO, 2017). This species has reduced home ranges, in early spring gilthead seabream migrates to estuaries and coastal lagoons searching for food and milder water temperatures. In autumn the fish migrates back to the sea to breed as adults or, as a very sensitive species to low temperature, simply to reach warmer sea waters when temperature drops in the shallow coastal waters (Abecasis & Erzini, 2008).

Figure 3 Gilthead seabream distribution

European production

Gilthead seabream has traditionally been farmed extensively in the coastal lagoons and brackish ponds of northern Italy and southern Spain. Early culturing of gilthead seabream was based on the capture of wild larvae and juveniles using fish barriers at lagoon entrances. Increased efforts were made to develop new intensive production practices as wild resources were reduced. In the 1980s, gilthead seabream was reproduced successfully in captivity and intensive rearing systems were developed, especially in sea cages and recently in land-based tanks (Moretti, Fernandez-Criado, & Vetillart, 2005). Continuing advancements in the nutritional and environmental requirements improved the survival and production rates, allowing expansion of the scale of production. Technical developments in the production system allowed the production of this species to increase rapidly in recent years. Robust nature, rapid growth, feeding habits and high-quality meat makes gilthead seabream a suitable specie for aquaculture and drives a high value market. However, increased production resulted in value reduction of market size gilthead seabream in the last years (Bartley, 1998). As the value of the product falls, substantial efforts are needed to maintain profits by increasing the efficiency of the production systems as well as developing new products and improving quality. The market for gilthead seabream is almost exclusively based on fresh sale and frozen whole fish. Therefore, the development of new products and expansion to new markets can undoubtedly change gilthead seabream production industry. Gilthead seabream is currently the only species of seabream which is farmed on a large scale and is the most important marine fish species produced in Europe. Gilthead seabream main producers in Europe are Greece, Spain and Italy, representing nearly 86% of total production. In 2017 European aquaculture production of gilthead seabream reached 92 998 tonnes and capture production merely 4 153 tonnes, representing only 4.3% of total production (Fig. 4) (FAO Fisheries and Aquaculture Department, n.d.).

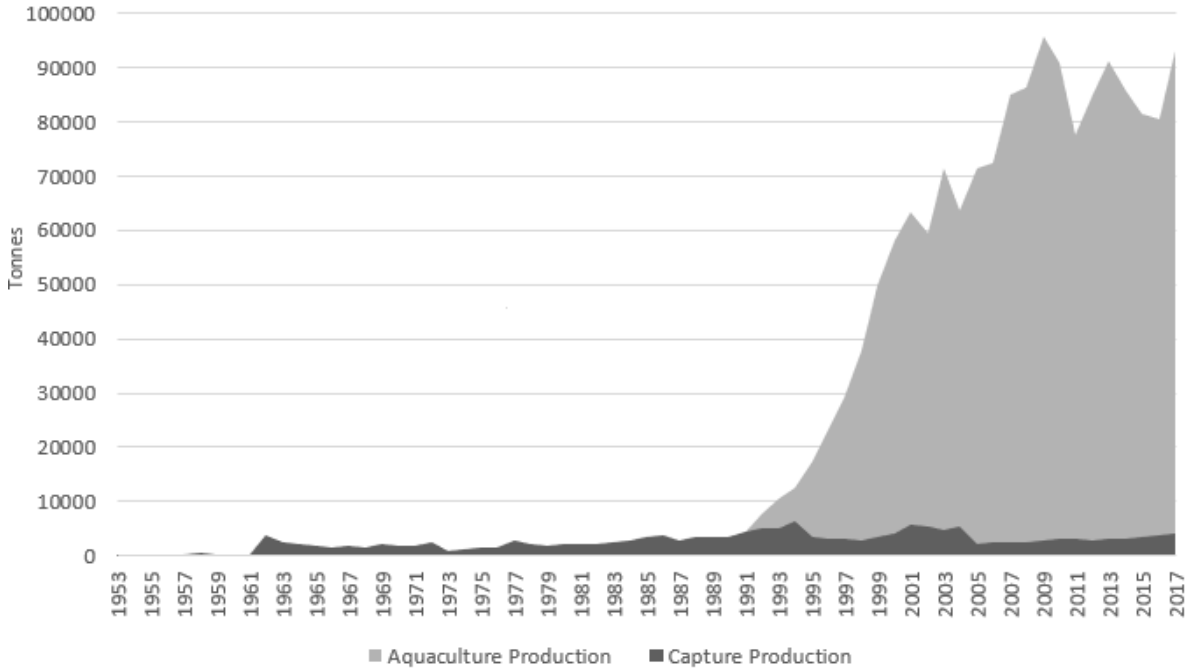

Figure 4 European capture and aquaculture production of gilthead seabream

Fish nutrition

Fish nutrition represents an extremely important research area in the aquaculture and aquafeed industry. Nutrition research involves the study of the essential nutrients required for the maintenance of life, the development and repair of body tissues and the production of energy to support biological functions (Hixson, 2014; Jobling, 2016). Fish nutrition research currently covers studies of physiological mechanisms involved in feed intake and its regulation, nutrient requirements and interactions, metabolic pathways, nutrient utilization and fish growth, reproduction and development. The study of nutritional influences on the ability of fish to resist environmental stressors and assemble an immune response under challenge from pathogens also forms part of fish nutrition research. Therefore, modern fish nutrition research involves a broad range of interrelated areas and often requires integration of knowledge gathered from advances in different sciences like chemistry, biochemistry, physiology, microbiology, immunology and molecular biology (Jobling, 2016). In aquaculture, good nutrition is fundamental to produce a healthy, high-quality fish product, and economically speaking is critical as feed typically represents approximately 50% of the

variable production costs (Craig & Helfrich, 2009). Fish nutrition has advanced dramatically recently in the production of balanced commercial diets and new species-specific formulations may support an expanding food production sector in an increasing demand for affordable, safe, high-quality fish and seafood products.

Nutritional requirements

Nutritionally, fish meal and fish oil are appropriate feed sources owing to their high protein and lipid content and high digestibility coefficients. They also possess an additional biological value due to their well-balanced essential amino acid and fatty acid composition. Moreover, elevated contents of essential highly unsaturated fatty acids (HUFAs), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), along with vitamins, as well as trace minerals, give them a high nutritional value. Nevertheless, fish meal and fish oil are unsustainable feed items, therefore, its replacement in aquafeeds by equally high nutritional, but environmentally sustainable and natural ingredients portray an important issue in aquaculture and aquafeed industry. As all animals, fish require essential nutrients like proteins, lipids, carbohydrates, vitamins and minerals for proper body function, thus diets should supply all the ingredients necessary for the optimal growth and health of fish. A well-balanced energy-to-protein ratio fish feed is extremely important to achieve an optimum diet with adequate nutrient content. A high energy relative to protein diet may result in excessive lipid deposition. Fish feed until their energy requirements are fulfilled, consequently, diets with high energy levels may lead to a decrease feed intake and reduced weight gain. In a similar way, a diet with insufficient energy content can equally result in reduced weight gain inasmuch the fish cannot eat enough feed to satisfy their energy requirements for growth (Craig & Helfrich, 2004). As has been said, formulating diets with an optimal nutrient balance is crucial in fish nutrition and production. Moreover, it is important to highlight that nutrition and fish production management should not only satisfy the dietary nutrient requirements of the farmed fish species for maximum growth, but also for increased immunocompetence and disease resistance (Hasan, 2001).

Fish do not have specific protein requirements in the diet but require specific amino acids which cannot be synthesized by the organism (Twibell & Wilson, 2002). Yet, research revealed protein intake requirement of 45% in juvenile gilthead seabream and 55% in fry for maximum growth (Martín et al., 1996; Robaina, 1996; Santinha, Gomes, & Coimbra, 1996).

Amino acids are organic compounds that form proteins. They are used to maintain existing proteins or to synthesize new ones, while excess protein is converted to energy. Around 200 amino acids occur in nature, but only about 20 amino acids are common in nutritional requirements. Of these, 10 are considered essential amino acids (EAA) that cannot be synthesized by fish, namely methionine, arginine, threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine, and phenylalanine. Of these, lysine and methionine are often considered limiting amino acids (National Research Council, 1993; Oliva-Teles, 2012; Twibell & Wilson, 2002). Research data on gilthead seabream quantitative amino acid requirements is available for arginine as 5 g/16g N (Luquet & Sabaut, 1974; Tibaldi, Lanari, Ballestrazzi, Tulli, & Pinosa, 1993; Tibaldi, Tulli, & Lanari, 1994), for lysine as 5 g/16g N (Luquet & Sabaut, 1974; Tibaldi & Lanari, 1991), for methionine and cysteine as 4 g/16g N (Luquet & Sabaut, 1974; Thebault, Alliot, & Pastoureaud, 1985) and for tryptophan as 0.6 g/16g N (Luquet & Sabaut, 1974). Alternative protein sources to fish meal are usually deficient in some essential amino acids. Therefore, it is important to know and provide the dietary protein and specific amino acid requirements to each fish species. Protein is the most expensive component of fish feed; thus, it is important to accurately determine the protein requirements for each species and life stage cultured. Protein requirements are generally higher during early life stages. As fish grow in size, their protein requirements usually decrease (Craig & Helfrich, 2009). In general, protein requirements are typically higher for carnivorous fish and vary with rearing conditions, water temperature and water quality, usually increasing for fish reared in high-density systems (e.g., recirculating aquaculture systems) (Craig & Helfrich, 2009). Protein requirement depends essentially on the bioavailability of the protein source, amino acids profile and dietary energy level, and is species-specific. Usually, protein is used for fish growth if adequate levels of lipids are present in the diet. However, if lipid content is lacking, fish use protein as an energy source to support biological functions rather than for growth purpose. Protein intake deficiency is recognized to impair immune function and increase fish susceptibility to infectious diseases along with reduced growth (P. Li, Gatlin, & Neill, 2007; P. Li, Yin, et al., 2007).

Lipids are high-energy nutrients conventionally used as energy source in fish diets (Halver & Hardy, 2002). Comparatively, lipids have about twice the energy density of proteins and carbohydrates (Craig & Helfrich, 2009). Dietary lipids typically provide essential fatty acids that cannot be synthesized by fish but are required for maintenance of cellular functions. Fish oil is preferably used as lipid source in aquafeed manufacture due to its high content in

essential fatty acids. Usually, high levels of lipids are used to reduce feed costs by partially sparing protein for growth (Halver & Hardy, 2002). However, high lipid levels can affect fish growth and body composition, often leading to an excessive fat deposition. Research in gilthead seabream growth revealed best performances with 15% lipid content in the diet using fish oil as lipid source (Robaina, 1996; Vergara & Jauncey, 1993). However, at a 9% dietary lipid content, the use of soybean oil as lipid source resulted in higher growth rates (Marais & Kissil, 1979). Fish have dietary requirements of n-3 and n-6 polyunsaturated fatty acids (PUFA), but marine fish generally have specific needs of n-3 fatty acids for optimal growth and health status. In gilthead seabream juveniles, research estimated essential fatty acids requirements of 0.9% highly unsaturated fatty acids (HUFA) in diet (Kalogeropoulos, Alexis, & Henderson, 1992), and best growth performances with an eicosapentaenoic to docosahexaenoic (EPA/DHA) ratio of 2:1 (Ibeas et al., 1997). Eicosapentaenoic acid (EPA: 20:5n-3) and docosahexaenoic acid (DHA: 22:6n-3) are the most important essential fatty acids in marine fish which intake is diet dependent. Essential fatty acids deficiency affect fish health in terms of immunosuppression and stress resistance, but also decreases complement, haemolytic and agglutination activity (Montero et al., 2003; Montero, Tort, Izquierdo, Robaina, & Vergara, 1998; Tort, Gómez, Montero, & Sunyer, 1996). Fatty acid composition of lipids varies depending on the source. Lipids from a marine source contain substantial amounts of long-chain polyunsaturated fatty acids which EPA and DHA are the two typical fatty acids present. Attention to the role of n-3 long-chain polyunsaturated fatty acids, specially EPA and DHA, in human health has been continuously increasing. In human, the major sources of EPA and DHA are marine products consumed in diet (Bhaskar & Miyashita, 2007). Several studies have shown n-3 fatty acids implications in infant development, cardiovascular diseases, several mental illnesses, including depression and Alzheimer's disease, and even cancer (Birch, Garfield, Hoffman, Uauy, & Birch, 2000; Caygill, Charlett, & Hill, 1996; Clandinin, Jumpsen, & Suh, 1994; du Bois, Deng, Bell, & Huang, 2006; Escrich, Solanas, Moral, Costa, & Grau, 2006; Fenton, Hibbeln, & Knable, 2000; Honig, 2000; Kimura et al., 2007; Kuriki et al., 2007; Theodoratou et al., 2007). Seafood and fish consumption in a regular basis could play an important role in the prevention and treatment of cardiovascular diseases, inflammatory and autoimmune disorders and cancer (Kris-Etherton, Harris, & Appel, 2002; Piepoli et al., 2016; S. et al., 2009; Saravanan, Davidson, Schmidt, & Calder, 2010; Simopoulos, 2003).

Carbohydrates are not essential nutrients in fish diet. Several studies demonstrated that fish

can use diets with no carbohydrates as efficiently as those including carbohydrates (Enes, Panserat, Kaushik, & Oliva-Teles, 2009; Hemre, Lambertsen, & Lie, 1991; Peres & Oliva-Teles, 2002; Sá, Pousão-Ferreira, & Oliva-Teles, 2007). However, carbohydrates can be mobilized to satisfy energy demands as they are stored in fish as glycogen. Carbohydrate utilization is related to source, molecule complexity, processing treatments, dietary inclusion level and it is species specific (Enes et al., 2009; Krogdahl, Hemre, & Mommsen, 2005; Stone, 2003; R. P. Wilson, 1994). Mostly, carbohydrates are used in aquafeeds to reduce costs and for its binding activity in diets manufacturing as it is a useful ingredient in manufacture floating feeds through extrusion process.

Vitamins are organic compounds necessary in trace amounts to support normal fish growth, reproduction and health (National Research Council, 1993). They must be provided in the diet because fish usually cannot synthesize them. However, in the presence of certain amounts of other essential nutrients some vitamins can be partially synthesized (Robert P Wilson & Poe, 1988). Vitamins can be characterized as water-soluble and fat-soluble. Water-soluble vitamins include vitamins B and vitamin C. Of these, vitamin C is considered the most import due to its powerful antioxidant properties, benefits to the immune system and its stress reduction effects in fish (Webster & Lim, 2001). Fat-soluble vitamins include vitamins A, vitamin D, vitamin E and vitamin K. Of these, vitamin E is the most studied regarding its important role as an antioxidant. However, research on the influence of vitamins on health and growth in fish remains limited (Cyrino, 2008). Nevertheless, considerable data have been accumulated showing that certain vitamins can improve stress tolerance, immunological response, disease resistance and even have antioxidant properties (Cyrino, 2008; Montero et al., 1999; Montero, Tort, Robaina, Vergara, & Izquierdo, 2001; Nakagawa, Sato, & Gatlin, 2007; Trenzado, Higuera, & Morales, 2007). Other distinct vitamin implications on metabolic functions have also been shown, specially antibody production and macrophage functions (Blazer, 1992; Halver & Hardy, 2002). However, those health benefits are vitamin level dependent and overfortification of diets is mandatorily required (Nakagawa et al., 2007; Webster & Lim, 2001).

Minerals are inorganic elements necessary in fish diet for normal body function. However, determination of dietary mineral requirement in fish is difficult as they can absorb minerals from the surrounding water body through their gills and skin in addition to the food ingested. Fish dietary requirement for a specific mineral depends largely on the concentration of the

element in the water (Hepher, 1988; Steffens, 1989). Minerals can be classified as macrominerals or microminerals based on the quantity required in the diet and the amount present in fish. Common macrominerals present in fish diet are calcium, sodium, chloride, potassium, chlorine, sulphur, phosphorous and magnesium. These minerals can regulate osmotic balance and aid in bone formation and integrity. Common microminerals are iron, copper, chromium, iodine, manganese, zinc and selenium. These trace elements are required in small amounts as components in enzymes and hormones. Mineral availability in fish feed is not always optimal to meet biological requirements. Fish feed may be rich in some minerals and deficient in others, moreover, some elements may be leached during feed processing (Hepher, 1988). Mineral requirement in fish is still poorly studied due to fish capacity to obtain these elements beyond diet (Halver & Hardy, 2002; National Research Council, 1993). Diet supplementation with certain mineral levels may enhance immune function and disease resistance in fish, although such effects are not always evident (Cyrino, 2008; Halver & Hardy, 2002; Lim, Webster, & Cheng-Sheng, 2008). Nevertheless, it is easy to prevent mineral deficiency problems in aquaculture as dietary supplementation is quite inexpensive (Webster & Lim, 2001).

Novel aquafeed materials

Currently, aquaculture and aquafeed industry uses fish meal and fish oil as main fish feed sources due to their high nutritional value, digestibility and palatability. However, its variable availability and cost compromise fish feed production and consequently aquaculture development. The replacement of the traditional fish meal and fish oil sources comprise not only the search for high nutritional value ingredients but also for environmentally sustainable solutions. Another issue of interest is the production of a natural, healthy and highly valuable market product that meets the specific consumer requirements. The substitution of marine-derived ingredients mainly fish meal and fish oil in aquafeeds by vegetable protein and oil sources can interfere with the fish nutritional profile. Vegetable ingredients are often characterized by low amounts of n-3 long-chain polyunsaturated fatty acids and poor iodine and selenium sources when compared to marine ingredients (Van Paemel, Dierick, Janssens, Fievez, & De Smet, 2010). The use of natural feed ingredients like algae and yeast biomass has instigated interest due to their high nutritional value and richness in bioactive compounds like these. Therefore, algae and yeast biomass were exploited as alternatives to fish meal and fish oil and as vehicles for bio-fortification of fish, with particular

interest in iodine, selenium and essential fatty acids.

Algae

Algae are photosynthetic organisms that grow in most aquatic habitats, including lakes, pounds, rivers and oceans. They can tolerate a wide range of temperatures, salinities, pH levels, different light intensities and even grow alone or in symbiosis with other organisms. Algae can be broadly classified as *Rhodophyta* (red algae), *Phaeophyta* (brown algae) and *Chlorophyta* (green algae) and classified by size as macroalgae or microalgae. Macroalgae are multicellular, large-size algae, visible with the naked eye, while microalgae are microscopic single cell organisms and may be prokaryotic, similar to cyanobacteria, or eukaryotic, similar to green algae (*Chlorophyta*). Algae produce a wide range of bioproducts, including proteins, lipids, carbohydrates, vitamins, pigments, bioactive compounds and antioxidants (Brennan & Owende, 2010). Microalgae biotechnology has gained considerable importance in recent decades and its use is extending into several biotechnological areas like nutraceutical research, renewable energy source, production of essential biomolecules, wastewater treatment, bioremediation and animal nutrition. Among all these, the use of algae as a source in nutrition and in the production of fish feed has triggered intensive research into finding natural ingredients that improve the quality of animal food products. Fish meal and fish oil have been used as main ingredients in the production of fish feed, however, decreasing supply and increasing costs of these resources threaten the sustainability and growth of the aquaculture industry. Therefore, complete or partial substitution of fish meal and fish oil with alternative sources is needed to solve this problem. Presently, algae are used worldwide as a successful alternative source with many applications. In fish feeding, microalgae have been used for increasing growth, feed utilization, physiological activity, stress response, disease resistance and carcass quality (Roy & Pal, 2015). Due to their high nutritional value, high growth rate, antioxidant property and disease resistance power, microalgae are increasing its popularity as an alternative source in aquaculture. Several microalgae contain high protein, lipid and carbohydrate content and are considered an important feed ingredient. Microalgae could be a plausible alternative protein source to fish meal due to their high protein content (30-45%) and richness in essential amino acids (Becker, 2007a; Brown, Jeffrey, Volkman, & Dunstan, 1997). Moreover, microalgae amino acid content compares favorably with that of other protein sources, giving them a considerable superior status in nutritional quality (Richmond,

1988). In general, microalgae lipid content range between 20-50% and contain essential fatty acids, fundamental in human and animal diet. Essential polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are usually present in high concentrations, therefore, making microalgae a considerable alternative lipid source in aquafeed industry (Borowitzka, 1995). Microalgae are also a good source of different vitamins (e.g. provitamin A, vitamin E and vitamin K) and several are present in high concentrations, which elevates their importance in aquaculture as a nutritional fish feed source (Brown, Mular, Miller, Farmer, & Trenerry, 1999; Cuellar-Bermudez et al., 2015). Carbohydrates are usually found in the form of starch, cellulose, sugars and other polysaccharides, playing an important role in algal biomass digestibility. Another interesting characteristic of microalgae is their vast range of pigment content like chlorophylls, carotenoids, phycobiliproteins and xanthophylls. Certain carotenoids exhibit nutritional and therapeutic relevance due to their ability to act as provitamin A, that is, they can be converted into vitamin A (García-González, Moreno, Manzano, Florencio, & Guerrero, 2005; Gouveia & Empis, 2003). Moreover, carotenoids have anti-inflammatory properties owing to their mitigating action on reactive oxygen species. Several studies have demonstrated that microalgae may enhance growth, nutrient utilization and gastrointestinal morphology, and improve survival rates and immune response in different fish species (Y. Li, Liang, Zhang, & Gao, 2016; Sarker, Gamble, Kelson, & Kapuscinski, 2016; Tibaldi et al., 2015; Vizcaíno et al., 2014). Due to rapid global expansion of the aquaculture industry, decreased access to fish meal and fish oil resulting from low availability of wild fish stocks and increased price, microalgae have become a particularly interesting alternative source in aquaculture feed standards. However, high production costs and relatively low mass production in an increasing sector like aquaculture and aquafeed industry compromise algae utilization in fish feed, as it still represents a controversial alternative to fish meal and fish oil (Becker, 2007b).

Laminaria

Laminaria is a genus of brown algae commonly called “kelp”. It is a large, tough and glossy alga which can grow from 1 to 3 meters in size and live for 3 to 6 years. *Laminaria* can be found in rock pools and attached to rocks, bedrocks or other hard substrate by spreading root-like protrusions called rhizoids in the lower intertidal and subtidal or sublittoral fringe, at a maximum depth of 20 meters. Its blade is large and shaped like a palm of a hand with

several more or less regular finger-like segments. The smooth and flexible stem is oval in cross-section and is usually free of epiphytes, although old stems which become slightly roughened may support a few epiphytes. *Laminaria* is well adapted to fast, turbulent water and multidirectional forces, characteristic to intertidal zones. Its distribution is limited to salinity, temperature, desiccation and general stress. *Laminaria* contains a variety of different substances mainly proteins and carbohydrates (Guiry & Guiry, 2019; Lamouroux, 1813). As an iodine-rich macroalgae, *Laminaria* could be used as main source of this mineral in feed formulation (Holdt & Kraan, 2011). High iodine content of *Laminaria* and its inclusion in fish feeds was shown to be a valuable way of increasing fish iodine composition in fresh water and marine fish species (Ramalho Ribeiro et al., 2015b; Schmid et al., 2003). In this context, *Laminaria* was used in this study as main source of iodine inclusion in diet formulations. Research provided general information on *Laminaria sp.* composition: 16.1% of protein, 2.4% of lipids and 39.3% of carbohydrates (% dry weight) (Kumar, Ganesan, Suresh, & Bhaskar, 2008; Mabeau & Fleurence, 1993).

Chlorella

Chlorella is a single-cell green algae belonging to the phylum Chlorophyta. It has a spherical shape, about 2 to 10 µm in diameter, and has no flagella. *Chlorella* contains green photosynthetic pigments namely chlorophyll *a* and chlorophyll *b* in its chloroplast. It reproduces asexually by non-motile reproductive cells, called autospores, that rupture through the mother cells. Its gel-like substance is composed by water, soluble proteins, vitamins and minerals and hosts internally mitochondria, a small nucleus and a Golgi body (Safi, Zebib, Merah, Pontalier, & Vaca-Garcia, 2014). Due to its rich composition in bioactive molecules *Chlorella* has been studied and used as a potential food product. *Chlorella* is one of the most common microalgae used in fish feed supplementation given its high protein content and noticeable improvement in fish growth and feed efficiency capabilities (Nakazoe, 1986; Shubert, Larsen, & Johnson, 1985). *Chlorella sp.* protein content represents approximately 50% of its weight and lipids around 10% with particular emphasis to n-3 fatty acids which represent nearly 35% of the lipids in *Chlorella*; fibers also represent an important percentage in *Chlorella* composition with values around 15% of its weight (Merchant, Phillips, & Udani, 2015; Morris, Almarales, Carrillo, & Bermúdez, 2008).

Schizochytrium

Schizochytrium is a spherical unicellular marine microalga which belongs to the family Thraustochytriacaea. It is a heterotrophic microorganism and is 10-20 μm in size. *Schizochytrium* produces biflagellate zoospore and the mature cells divided by repeated binary division. Each *Schizochytrium* cell could develop into a sporangium that produces several zoospores (Kamlangdee & Fan, 2003). Certain species produce large amounts of docosahexaenoic acid (DHA) and are grown commercially for the production of this lipids for animal feeds. *Schizochytrium sp.* lipid content can reach values of around 66% and can produce up to 40% of DHA, accounting for its total production of fatty acids (Allen et al., 2019; Ashford, Barclay, Weaver, Giddings, & Zeller, 2000).

Tetraselmis

Tetraselmis is a unicellular photosynthetic green algae that lives in water but can also inhabit the surrounding land surface that are washed into the aquatic environment. Some species occur in plankton, others are benthic, colonizing sand, and a few occur as endosymbionts in metazoans (Provasoli, Yamasu, & Manton, 1968; Serôdio, Silva, Ezequiel, & Calado, 2011). Cells can assume cordiform, elliptical or almost spherical form and the cell body is covered by a solid cell wall called theca (Manton & Parke, 1965). *Tetraselmis* is a unicellular flagellate organism with four equal flagella in two opposite pairs. It contains a single cup-shaped chloroplast, very rarely observed two chloroplasts, usually with a central pyrenoid. It reproduces asexually by binary division in the non-motile stage. Due to its high lipid and protein content, approximately 50% and 40% (% dry weight), respectively, *Tetraselmis* has been considered a highly promising feed ingredient (Dammak et al., 2016; Fabregas & Herrero, 1985).

Iodine

Iodine is an essential micronutrient incorporated exclusively through diet (Niwattisaiwong, Burman, & Li-Ng, 2017). This element is fundamental for the synthesis of thyroid hormones important in metabolism regulation, promoting growth, development and maturation of organs, especially the brain, as it is crucial for neurodevelopment. Iodine deficiency can

lead to a number of health outcomes throughout life such as goiter, hypothyroidism, intellectual impairment, impaired physical development, increased risk of perinatal death and abortion, collectively termed iodine deficiency disorders (IDD) (B. Hetzel, 1983; Zimmermann, 2009). Although iodine deficiency can lead to a broad spectrum of disorders throughout life, it is most critical in the early stages of development, as the fetal brain is extremely dependent on iodine supply. During pregnancy, iodine requirement is enhanced due to the increase in maternal thyroxine (T4) production, iodine transfer to the fetus and increase in renal iodine clearance (Zimmermann, 2012). It is generally accepted that damage to the brain resulting in mental retardation is the most significant effect of iodine deficiency (M. Li & Eastman, 2012). On a global scale, almost 30% of the children at school age have a low iodine intake, and recent research revealed a mild iodine deficiency in pregnant females in some countries throughout the globe, such as United States of America (Caldwell et al., 2013), Italy (Mian et al., 2009), Norway (Brantsæter, Abel, Haugen, & Meltzer, 2013), United Kingdom (Bath, Steer, Golding, Emmett, & Rayman, 2013; Bath, Walter, Taylor, Wright, & Rayman, 2014) and Portugal (Limbert et al., 2010). Human intake of iodine is mainly from food, with some populations also obtaining appreciable quantities of iodine from drinking water. Most of the continental areas worldwide are lacking of iodine in the natural food chain, and iodine supplementation is crucial to prevent iodine deficiency disorders (Andersson, M., De Benoist, B., Darnton-Hill, I., & Delange, 2007; Andersson, Karumbunathan, & Zimmermann, 2012; B. S. Hetzel, 2012; Zimmermann, 2009). World Health Organization (WHO), United Nations Children's Fund (UNICEF) and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) recommend an intake of 150 µg iodine/day for adults and 220-290 µg/day for pregnant and lactating women. In children, iodine daily intake recommendation depends on age, 120 µg/day for children 6 to 12 years and 90 µg/day for infants and children up to 5 years of age (Zimmermann 2008; Andersson et al. 2007). Dairy products and iodized salt have been used as a vehicle for fortification and increase iodine intake. However, salt reduction programs have compromised iodized salt as a mean of incorporation. Fish and seafood are considered a natural source of iodine in food, thus suggested as a suitable vehicle for iodine fortification and an alternative to iodized salt (Haldimann, Alt, Blanc, & Blondeau, 2005; Institute of Medicine (US), 2000; World Health Organization, 2014). In fish, iodine can be taken up not solely from diet but also from the surrounding water via the gills. As seawater contains more iodine than freshwater, iodine intake is generally lower in freshwater fish (Hunn & Fromm, 1966). Iodine is related to thyroid hormones which regulate cellular oxidation,

neuromuscular control, circulatory dynamics, nutrient metabolism and growth. Triiodothyronine (T3) is the active thyroid hormone and thyroxine (T4) its precursor. Fish differ from mammals in iodine utilization and in the extrathyroidal metabolism of T3 and T4, as T3 binds more strongly to plasma proteins than T4 (Higgs, Fagerlund, Eales, & McBride, 1982). Most of the circulating T3 is provided by iodothyronine deiodinase 1 (*dio1*) gene expression which encodes a protein that catalyzes the activation and posteriorly the conversion of the T4, secreted by the thyroid gland, to the bioactive T3 (Itoh, Watanabe, Wu, & Suzuki, 2010). Some research noted that iodine intake and uptake may vary from surrounding water but also from fish diet (Agrawal & Mahajan, 1981; Woodall & LaRoche, 1964). Although iodine requirement of most fish species has not been established, a minimum dietary level of 2.8 $mg.kg^{-1}$ of iodine is recommended. European Union legislation authorizes a maximum content of 20.0 $mg.kg^{-1}$ of complete feed for farmed fish (European Food Safety Authority, 2005). However, on the limited available data, farmed fish tolerance levels of iodine are considered to be higher than 60.0 $mg.kg^{-1}$ of feed (European Food Safety Authority, 2005). It is considered that age, physiological state and stress factor may influence fish iodine requirement. In fish feeds, fish meal is considered the main source of iodine. Formulated fish diets with fish meal incorporation reduction and concomitant use of vegetable ingredients should be supplemented with an iodine rich source (Lovell, 1989).

Selenium

Selenium is an essential trace element that exerts multiple and complex effects in human and animal health due to its potent antioxidant, anti-inflammatory and antiviral properties. Its physiological functions are carried out by selenoproteins in which selenium is incorporated as the amino acid selenocysteine (Jr., MacDonald, & Zeisel, 2012). A low intake in selenium can cause physiological dysfunction and can make the organism more susceptible either to infection or environmental stressors. In livestock, selenium enriched products have been proposed as dietary supplements to be included into functional feeds for animal preventive health care. Selenium supplementation in farmed animal feed may reduce problems caused by deficiency of this mineral but also enhance physiological response to infection, inflammatory disorders and stress. Particularly in fish, research has demonstrated that selenium deficiency intake can cause elevated mortality, depressed feed efficiency and growth, and increase oxidative stress and haemolytic rates (Khan et al., 2016; Lin & Shiau, 2005). Several studies reported that selenium supplementation increase fish growth by

promoting deiodinase synthesis (Khan, Zuberi, Fernandes, Ullah, & Sarwar, 2017). This enzyme has a regulatory function capacity to convert an inactive thyroid hormone (thyroxine, T4) into a metabolically active thyroid hormone (triiodothyronine, T3). Elevated serum T3 concentration consequently raise growth hormone messenger RNA levels in the pituitary cells and stimulate the synthesis of growth hormone in fish, thus increasing fish growth (Farchi-Pisanty, Sternberg, & Moav, 1997; Khan et al., 2016; Khan, Zuberi, Nazir, et al., 2017; Sternberg & Moav, 1999). Another important selenoprotein is glutathione peroxidase (GPx) which regulates oxidative processes and cell membrane protection by catalyzing the reduction of hydroperoxidaes and lipid peroxidase (Brigelius-Flohé & Maiorino, 2013; Papp, Lu, Holmgren, & Khanna, 2007). However, selenium supplementation is still controversial because it has a narrow range between nutritive requirements and toxicity levels. Nutritional requirements may also vary considerably between species and physiological conditions within an organism, and even environmental factors can influence the dietary needs for this element. To this end, it is important to understand its bioavailability form, optimal range of concentrations for supplementation and its impact in the organism. In animal feed industry, selenium supplementation has been generally provided as sodium selenite (Na_2SeO_2), an inorganic form, or as selenomethionine (Se-Met), an organic form. Sodium selenite is the most used inorganic selenium supplement in animal food due to its easy supplementation as a stable and high soluble salt. As an organic supplement, selenomethionine is the preferred selenocompound used for selenium augmentation. However, selenomethionine must be synthetized by organisms such as fungi and plants given the impossibility of being produced by animals. Previously, selenium supplementation in animal feeds primarily focused on inorganic selenium, mainly sodium selenite as explained (Pacitti et al., 2015; Vignola et al., 2009), regardless its low concentration tolerance and reduced bioavailability. However, some new selenium enriched products are being studied and used, such as selenium-yeast. Selenium-yeast is a mixture of selenocompounds produced by yeast, mainly *Saccharomyces cerevisae*, that is exposed to sodium selenite. This new product could possibly deliver selenium in a more natural form of selenium-methionine, as it is the most abundant selenocompound, representing roughly 70% of the total mass. It is believed that selenium-yeast is highly bioavailable and consequently it can be tolerated at higher concentrations and so applied in feed supplementation more efficiently (Rayman, 2004). The European Union has legislated a maximum limit value for total selenium of 0.5 $mg.kg^{-1}$ (dry mass) of feed with a maximum supplementation with organic selenium of 0.20 mg $Se.kg^{-1}$ of

complete feed with a moisture content of 12% (The European Comission, 2013). However, these values are based on researches using inorganic selenium like sodium selenite. Recent studies suggest that organic selenium is more bioavailable and tolerated at higher concentrations than inorganic selenium (Thiry, Ruttens, De Temmerman, Schneider, & Pussemier, 2012). In accordance with these studies, it is mandatory to perform new research using organic forms as supplement in order to update these legislation values. Selenium supplementation can have beneficial effects on fish health and welfare, giving exogenous feeding in aquaculture the possibility to tailor fish body composition in terms of bioactive compounds. In this context, formulation of functional foods and feed additives research have become an area of interest in aquaculture industry.

Functional Food

Diet primary role is to provide sufficient nutrients such as proteins, lipids, carbohydrates, minerals and vitamins to meet nutritional requirements of an individual. Some research has provided scientific evidence to support the hypothesis that some foods or food components have beneficial effects over the provision of the basic nutrients obtained from diet (Finley, 2009; Palou, Oliver, Rodríguez, & Caimar, 2007). Nutrition research focus nowadays more in the identification of biologically active compounds in foods that have the potential to improve health and well-being and which may also have some reductive effect in risk disease. In addition, new food products are being developed to enhance or even incorporate these beneficial components and thus to provide consumers with associated beneficial effects (Finley, 2009). However, functional foods have not been defined by legislation in Europe. Generally, they are considered as foods that are intended to be consumed as part of a normal diet and that contain biologically active components which offer the potential to enhance health or reduce risk of disease. Biological compounds included in functional foods are normally specific fatty acids, minerals and vitamins. Functional Food Center (FFC) defines functional foods as: “Natural or processed foods that contain biologically active compounds; which, in defined, effective, and non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers for the prevention, management, or treatment of chronic disease or its symptoms” (Gur, Mawuntu, & Martirosyan, 2018). A functional food can be a natural or processed food to which a component has been added or removed by technological or biotechnological means. Thus, the inclusion of functional foods in diet may be a close approach to support

increased intake of essential nutrients as biofortification could represent a sustainable and beneficial intervention in consumers health, well-being and disease prevention if properly applied and regulated (Bouis & Saltzman, 2017). As legislation and regulation increases, functional foods and consequent biofortification of food products will consolidate its importance in food industry and diet (Luthringer, Rowe, Vossenaar, & Garrett, 2015).

The present study aims to find alternative sources to fish meal and fish oil, not only to provide insight of a sustainable solution for the aquaculture industry and the environment, but also to ensure the production of a high nutritional value product to the consumers.

Materials and Methods

Pilot scale-trial

A pilot scale-trial with gilthead seabream was carried out in Olhão, Portugal, at the aquaculture research facility EPPO-IPMA. The trial was performed between July and October for 10 weeks in an open seawater system equipped with twelve 3 m^3 tanks in a 6 $kg.m^{-3}$ fish density. System was supplied by a continuous water flow at 25.2 ± 1.4 °C temperature and 5.6 ± 0.9 $mg.L^{-1}$ dissolved oxygen. Photoperiod was naturally provided and light conditions similar in all tanks. Diets were randomly assigned in triplicate tanks and fish were fed 1.3-2% biomass by hand four times a day, six days a week. Homogenous groups of fish with a mean body weight of 370 g were established and randomly distributed to each tank.

Experimental diets

Gilthead seabream were fed with four isonitrogenous (50% DM) and isoenergetic (24 $kJ.g^{-1}$ DM) diets presented in **Table 1**: a commercial-based diet (FMFO) and three experimental diets with a 33% replacement of fish meal by a microalgae biomass (*Chlorella, Tetraselmis* and *Schizochytrium*) (AB diets). A further replacement of 20% FO was tested in ABVO diet. All AB rich diets were supplemented with both *Laminaria* and Se-yeast: diets $ABVO_{I8+Se1}$ and $ABFO_{I8+Se1}$ provided equivalent levels of iodine (8 $mg.kg^{-1}$) and selenium (1 $mg.kg^{-1}$),

whereas an extreme diet $ABFO_{I15+Se1.4}$ was further enriched with iodine (15 $mg.kg^{-1}$) and selenium (1.4 $mg.kg^{-1}$).

Table 1 Experimental diets composition

FMFO	Composition similar to analyzed commercial feed
$ABVO_{I8+Se1}$	33% replacement of fishmeal mainly by microalgae (*Chlorella* and *Tetraselmis*) 20% replacement of fish oil with DHA-rich algae *Schizochytrium* Supplementation with DHA-rich algae *Schizochytrium* to provide EPA+DHA levels identical to CTRL (11 $mg.g^{-1}$) Iodine level, provided by Laminaria, at 8 $mg.kg^{-1}$ Selenium level, provided by Se-yeast, identical to CTRL (1 $mg.kg^{-1}$)
$ABFO_{I8+Se1}$	33% replacement of fishmeal mainly by microalgae (*Chlorella* and *Tetraselmis*) No replacement of fish oil Supplementation with DHA-rich algae *Schizochytrium* Iodine level, provided by *Laminaria*, at 8 $mg.kg^{-1}$ Selenium level, provided by Se-yeast, identical to CTRL (1 $mg.kg^{-1}$)
$ABFO_{I15+Se1.4}$	33% replacement of fishmeal mainly by microalgae (*Chlorella* and *Tetraselmis*) No replacement of fish oil Supplementation with DHA-rich algae *Schizochytrium* Iodine level, provided by *Laminaria*, at 15 $mg.kg^{-1}$ Selenium level, provided by Se-yeast, at 1.4 $mg.kg^{-1}$

Fish sampling

Fish were bulk weighted at the beginning and by the end of the trial after 24 hours of feed deprivation. At the end of the trial 6 fish per tank, totaling 18 fish per treatment, were anaesthetized, sacrificed and biometry measurements like weight, fork length (FL) and total length (TL) were performed. Posteriorly, fish were stored at -20°C for posterior proximate whole-body composition analysis. Another 5 fish per tank, a total of 15 fish per treatment, were anaesthetized, measured and sacrificed. Fish were then dissected, total viscera and individual organs weighted, and liver and muscle were collected for later analysis. After collected, tissues were immediately fast-frozen in liquid nitrogen and stored at -80°C.

Chemical analysis

Proximate Composition

Prior to the proximate composition analyzes, fish from each tank were minced, pooled and subsequently freeze dried. Proximate composition of gilthead seabream whole-body from the four groups was determined according to AOAC procedures. Ash (incinerated at 500°C for 5 hours) according to 942.05 method, dry matter (in oven at 105°C to constant weight) was determined according to 934.01 method, protein by quantitation of nitrogen in N_2 form by a microprocessor (Leco FP-528) and conversion (N x 6.25) to equivalent protein according to 990.03 method and total lipids by homogenization of the sample in organic solvent and subsequent evaporation of the mixture for concentration of lipid extracts according to Folch Method (Folch, Lees, & Stanley, 1957).

RNA extraction and cDNA synthesis

Liver and muscle samples were disrupted with NZYol reagent (NZYTech, Lda.) using Precellys 24 Tissue Homogenizer (Bertin Instruments). Total RNA was extracted using NZYTech Total RNA Isolation Kit (NZYTech, Lda.) following the protocol. RNA quality was verified in 1% TAE (w/v) agarose electrophoreses gel stained with GelRed®. cDNA was synthesized from total RNA using NZYTech Fist-Strand cDNA Synthesis Kit (NZYTech, Lda.) following the protocol.

Real-Time PCR

Expression of 10 genes, including carnitine palmitoyltransferase 1a (*cpt1a*), deiodinase 1 (*dio1*), glutathione peroxidase 1a (*gpx1a*), glutathione peroxidase 1b (*gpx1b*), glutathione peroxidase 4b (*gpx4b*), liver X receptor a (*lxra*), fatty acid elongase 5 (*elovl5*), fatty acid desaturase 2 (*fads2*), insulin-like growth factor 1 (*igf1*) and insulin-like growth factor 2 (*igf2*) were determined by quantitative real-time PCR using specific real-time PCR primers (Table 3). Real Time PCR was performed in Mastercylcer® EP Realplex (Eppendorf AG) using NZYSpeedy qPCR Green Master Mix (2x) (NZYTech, Lda.). Samples were prepared to a total of 10 µL reaction volume with 2 µL of cDNA, 2.2 µL of ultra-pure water, 0.8 µL of primer

(forward and reverse) and 5 µL of NZYSpeedy qPCR Green Master Mix (2x). Thermal cycling was performed as follows: initial polymerase activation at 95°C for 2 minutes; 40 amplification cycles for denaturation at 95°C for 5 seconds and annealing process for 28 seconds at gene specific temperature (Table 2). Melting curve was generated to verify specificity of the assays. To evaluate the relative transcript levels, the $2^{-\Delta\Delta CT}$ method was used with ***ef1α*** and *s18* in liver and ***β-actin*** and *s18* in muscle as best housekeeping genes, estimated by geNorm® software to provide the most reliable normalization. The PCR efficiency for target genes ranged from 90% to 108%.

Table 2 Gene primers used in real-time PCR

Gene	Primer sequence (5' – 3')	Annealing T. (°C)	Efficiency		Acession number
			Liver	Muscle	
β-actin	F - GTGCCTTCGTTCGTTCCATGATC	58°C	0.91	0.99	X89920.1
	R - TGATGCTTATCTGCTGCCTGTTTG				
ef1α	F - GACTCGTCAGGGACTTCAGC	60°C	1.00	0.93	AF184170.1
	R - GAGCCTCTCAGGCATAGCAC				
18S	F - CGACAACTACCCACCAACCT	60°C	1.01	1.12	AM490061.1
	R - AATTGTAGCCGCCATACCAC				
cpt1a	F - TGGAGAAGGTGGATGTGAATG	58°C	0.99		JQ308822.1
	R - GCCATCAGGACCAACAAGG				
dio1	F - GCGTTACAGCAGGAGGTT	60°C	0.97		DQ888894.1
	R - TGAGCACATCTGGTCATTCA				
elovl5	F - ACGGCTTGTCTGTAAACTC	60°C		1.08	GQ162823.1
	R - GCTGGTGAATGTCCTCTC				
fads2	F - TGTCTAGCGCTCTTTCCTTTCA	60°C	0.99		AY055749.1
	R - AGAGGGTGTGGCTACAGGAGATAC				
gpx1a	F - TGGGATCGTAGAGGAGTGTTGT	58°C		1.01	KC201252.1
	R - CTGTAGAGAGGTGGCCGACA				
gpx1b	F - GCACTTCGCCTCCAGGACAAG	60°C	0.90		KC201353.1
	R - CAGTCTTCACACAGCCACATCAGG				
gpx4b	F - CGCCGTGGACCTGTCAGAGC	60°C	0.99	0.99	KC201354.1
	R - GGAATGGATGGAGGAGGAGGAGATGG				
igf1	F - GTGCCTTCGTTCGTTCCATGATC	60°C		1.01	AY996779.2
	R - TGATGCTTATCTGCTGCCTGTTTG				
igf2	F - GACTCGTCAGGGACTTCAGC	62°C		0.95	AY996778.1
	R - GAGCCTCTCAGGCATAGCAC				
lxra	F - CGACAACTACCCACCAACCT	60°C	0.98		FJ502320.1
	R - AATTGTAGCCGCCATACCAC				

Statistical Analysis

Statistical analysis was performed using IBM® SPSS® Statistics 25 software. Statistic evaluation of the data was accomplished by one-way analysis of variance (ANOVA). All variables were checked for normality and homogeneity of variance, by using the Shapiro-

Wilk and the Levene test, respectively. Significant differences between means were calculated through Tukey's multiple comparison test. A significance of $p < 0.05$ was applied to all statistical tests. Correlation analysis was performed using Spearman's correlation test. A probability of $p < 0.05$ was considered significant.

Results

Growth performance

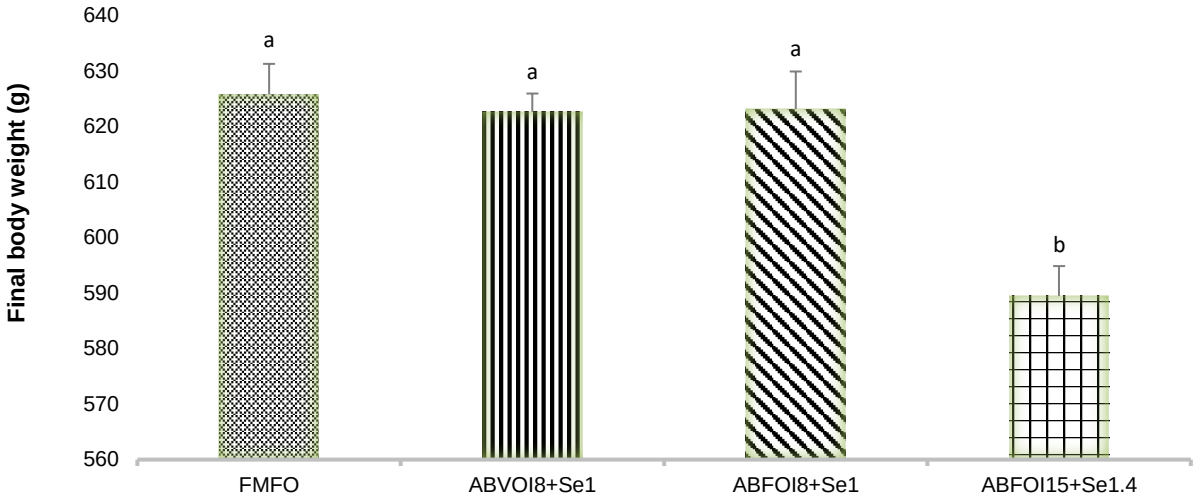

Figure 5 Final body weight (g) of gilthead seabream fed the experimental diets

Final body weight of gilthead seabream fed with biofortified diets $ABVO_{I8+Se1}$ (622.61 ± 6.77 g) and $ABFO_{I8+Se1}$ (623.11 ± 3.29 g) was similar to those fed with FMFO (625.76 ± 5.38 g) diet, with exception of $ABFO_{I15+Se1.4}$ (589.46 ± 5.49 g) diet that resulted in a significant lower weight (Fig. 5). Feed conversion ratio (FCR) did not differ significantly between diets. Also, specific growth rate (SGR) did not present significantly differences between dietary treatments. Protein efficiency ratio (PER) showed significant differences between diets, with values in fish fed $ABFO_{I15+Se1.4}$ (0.93 ± 0.07) diet lower than in fish fed FMFO (1.10 ± 0.02) diet. Hepatosomatic index (HSI) also presented significantly differences between diets, with fish fed FMFO (1.56 ± 0.02) diet showing higher values than those fed $ABVO_{I8+Se1}$ (1.31 ± 0.09) and $ABFO_{I8+Se1}$ (1.29 ± 0.10) diets (Table 3).

Table 3 Fish growth performance

	Dietary treatments			
	FMFO	**ABVO$_{I8+Se1}$**	**ABFO$_{I8+Se1}$**	**ABFO$_{I15+Se1.4}$**
Initial body weight (g)	371.20 ± 14.50	378.95 ± 2.38	375.52 ± 13,39	369.07 ± 9.82
Final body weight (g)	625.76 ± 5.38 a	622.61 ± 6.77 a	623.11 ± 3.29 a	589.46 ± 5.49 b
Specific growth rate (SGR)	0.61 ± 0.04	0.58 ± 0.02	0.60 ± 0.05	0.55 ± 0.04
Feed conversion ratio (FCR)	1.86 ± 0.03	1.89 ± 0.07	1.96 ± 0.15	2.20 ± 0.25
Protein efficiency ratio (PER)	1.10 ± 0.02 a	1.05 ± 0.04 ab	1.02 ± 0.07 ab	0.93 ± 0.07 b
Condition index (KI)	1.81 ± 0.02	1.99 ± 0.18	1.82 ± 0.07	1.76 ± 0.15
Hepatosomatic index (HSI)	1.56 ± 0.02 a	1.31 ± 0.09 b	1.29 ± 0.10 b	1.34 ± 0.11 ab
Viscerosomatic index (VSI)	5.70 ± 0.18	5.60 ± 0.25	5.75 ± 0.83	5.73 ± 0.15

Final body composition and gain

Protein, lipids and energy final body composition and gain were not significantly affected by dietary treatments (Table 4).

Table 4 Fish final body composition and gain

	Dietary treatments			
Final body composition (% wet weight) *	**FMFO**	**ABVO$_{I8+Se1}$**	**ABFO$_{I8+Se1}$**	**ABFO$_{I15+Se1.4}$**
Humidity (%)	59.61 ± 0.66	60.14 ± 0.12	60.63 ± 0.95	59.87 ± 0.96
Ash (%)	3.43 ± 0.02	3.63 ± 0.07	3.63 ± 0.26	3.70 ± 0.18
Protein (%)	18.07 ± 0.46	18.35 ± 0.03	18.61 ± 0.30	18.18 ± 0.40
Lipids (%)	15.27 ± 0.43	15.69 ± 1.28	14.67 ± 1.23	14.52 ± 0.84
Energy (kJ.g^{-1})	10.94 ± 0.32	11.11 ± 0.43	10.70 ± 0.35	11.02 ± 0.37
Gain (g or kJ or Mg.kg^{-1} ABW day^{-1})				
Dry matter (g)	2.82 ± 0.24	2.63 ± 0.05	2.60 ± 0.10	2.54 ± 0.26
Protein (g)	1.10 ± 0.04	1.09 ± 0.03	1.15 ± 0.12	1.00 ± 0.06
Lipids (g)	1.14 ± 0.11	1.02 ± 0.17	1.02 ± 0.14	0.94 ± 0.15
Energy (kJ)	0.79 ± 0.08	0.79 ± 0.05	0.74 ± 0.05	0.74 ± 0.09

* Initial body composition (% or kJ.g^{-1} of wet weight) was: humidity 64.02, protein 12.78, fat 16.3, ash 4.32 and energy 9.41.

Gene expression in liver and muscle

The expression of *dio1*, *fads2*, *gpx1b*, *gpx4b* and *lxra* in liver did not present significantly differences between dietary treatments. However, *cpt1a* expression varied significantly between diets, with fish fed $ABVO_{I8+Se1}$ (2.1 ± 0.31) diet showing an upregulation of *cpt1a* compared to all other dietary treatments (Table 7).

Table 5 Gene expression in liver

Gene	Liver			
	FMFO	**$ABVO_{I8+Se1}$**	**$ABFO_{I8+Se1}$**	**$ABFO_{I15+Se1.4}$**
cpt1a	1.0 ± 0.1 b	2.1 ± 0.3 a	0.7 ± 0.1 b	1.4 ± 0.2 b
dio1	1.0 ± 0.2	1.4 ± 0.3	1.5 ± 0.3	1.1 ± 0.2
fads2	1.0 ± 0.3	1.4 ± 0.2	2.1 ± 0.8	2.4 ± 0.5
gpx1b	1.0 ± 0.2	0.7 ± 0.1	1.0 ± 0.3	0.8 ± 0.2
gpx4b	1.0 ± 0.2	2.2 ± 0.5	2.5 ± 0.6	1.4 ± 0.3
lxra	1.0 ± 0.1	1.5 ± 0.2	1.0 ± 0.1	1.0 ± 0.1

cpt1a, carnitine palmitoyltransferase 1a; *dio1*, deiodinase 1; *fads2*, fatty acid desaturase 2; *gpx1b*, glutathione peroxidase 1b; *gpx4b*, glutathione peroxidase 4b; *lxra*, liver X receptor a.
Values are presented as mean ± se (n = 9 fish per treatment).

In muscle, the expression of *elovl5*, *gpx4b*, *igf1* and *igf2* did not show significantly differences between diets. Nevertheless, *gpx1a* showed higher abundance in fish fed $ABFO_{I15+Se1.4}$ (2.5 ± 0.5) diet compared to fish fed FMFO (1.0 ± 0.2) (Table 8).

Table 6 Gene expression in muscle

Gene	Muscle			
	FMFO	**$ABVO_{I8+Se1}$**	**$ABFO_{I8+Se1}$**	**$ABFO_{I15+Se1.4}$**
elovl5	1.0 ± 0.1	2.3 ± 0.8	3.8 ± 1.4	1.0 ± 0.3
gpx1a	1.0 ± 0.2 b	2.3 ± 0.4 ab	2.1 ± 0.2 ab	2.5 ± 0.5 a
gpx4b	1.0 ± 0.1	1.3 ± 0.3	1.3 ± 0.1	1.2 ± 0.3
igf1	1.0 ± 0.3	1.6 ± 0.5	0.9 ± 0.2	1.8 ± 0.5
igf2	1.0 ± 0.1	1.6 ± 0.2	1.6 ± 0.2	1.3 ± 0.2

elovl5, fatty acid elongase 5; *gpx1a*, glutathione peroxidase 1a; *gpx4b*, glutathione peroxidase 4b; *igf1*, insulin-like growth factor 1; *igf2*, insulin-like growth factor 2.
Values are presented as mean ± se (n = 7 fish per treatment).

Correlation between growth performance and gene expression

Gene expression of carnitine palmitoyltransferase 1a (*cpt1a*), deiodinase 1 (*dio1*), fatty acid desaturase 2 (*fads2*), glutathione peroxidase 1b (*gpx1b*), glutathione peroxidase 4b (*gpx4b*) and liver X receptor a (*lxra*) in liver and fatty acid elongase 5 (*elovl5*), glutathione peroxidase 1a (*gpx1a*), glutathione peroxidase 4b (*gpx4b*) insulin-like growth factor 1 (*igf1*) and insulin-like growth factor 2 (*igf2*) gene expression in muscle presented no correlation with fish growth performance.

Discussion

Aquaculture is a thriving and important food supply sector worldwide, and yet is capable of much more expansion and improvement. Its deployment relies mostly on the industry capacity to develop efficient production methods and in the production of sustainable and high nutritional value products. The replacement of the traditional fish meal and fish oil as aquafeed sources is crucial due to its unstable availability and price. Algae as a nutritional source have been widely used in animal nutrition as an alternative source replacing fish meal and fish oil successfully. In fish feeding trials, many types of algae have been used for increasing growth, feed utilization, physiological activity, stress response, disease resistance and general carcass quality (Nakagawa, 1993, 1997; Satob, Nakagawa, & Kasahara, 1987; Valente et al., 2006; Wassef, El Sayed, Kandeel, Mansour, & Sakr, 2005). Algae incorporation in formulated diets can result not only in an improved final body weight and feed conversion ratio but also in accumulated DHA and total n-3 fatty acids content in fish muscle (M. H. Li, Robinson, Tucker, Manning, & Khoo, 2009; Miller, Nichols, & Carter, 2007). In this context, natural feed ingredients like macroalgae, microalgae and yeast biomass were exploited as alternative feed sources. The present research showed some promising results in the use of algae as fish feed ingredients in terms of fish growth performance. Final body weight of fish fed the biofortified $ABVO_{I8+Se1}$ and $ABFO_{I8+Se1}$ diets was similar to fish fed FMFO diet, with the exception of fish fed $ABFO_{I15+Se1.4}$ diet, which resulted in a significantly weight reduction. These results highlight the possibility of replacing fish meal and fish oil with alternative sources such as algae biomass without affecting growth performance. Likewise, the dietary inclusion of 10% *Laminaria digitata* in gilthead

seabream (Ramalho Ribeiro et al., 2015a), or 10% *Porphyra dioica* in formulated diets for rainbow trout (Soler-Vila, Coughlan, Guiry, & Kraan, 2009) had no implications in growth. Improved growth performance was observed in red seabream fed diets with 5% of *Porphyra yezoensis* (Mustafa, Wakamatsu, Takeda, Umino, & Nakagawa, 1995) and in gilthead seabream with *L. digitata* included in feed (Ramalho Ribeiro et al., 2017). However, high inclusion of algae in fish feeds may have negative results. The dietary inclusion of 15% *L. digitata* resulted in a significant weight loss in rainbow trout (Soler-Vila et al., 2009). Similarly, in the present study, the highest inclusion of *Laminaria sp*. ($ABFO_{l15+Se1.4}$ diet) resulted in a lower final body weight in gilthead seabream. This also resulted in the lowest protein efficiency ratio (PER), confirming previous observations in gilthead seabream fed a diet with 10% *L. digitata* (Ramalho Ribeiro et al., 2015a). Although not significant, feed conversion ratio (FCR) tended to increase in fish fed diets with *Laminaria sp.*. This may be partially explained by the high algae content in soluble and insoluble polysaccharides, resulting in a faster passage of feed through fish digestive tract resulting in increased FCR values (Oliveira et al., 2009). The dietary inclusion of algal biomass resulted in lower hepatosomatic index (HSI) values in fish fed $ABVO_{l8+Se1}$ and $ABFO_{l15+Se1.4}$ diets which might be explained by a reduced deposition of lipids in liver (Azaza et al., 2008; Güroy, Ergün, Merrifield, & Güroy, 2012). However, without significant effects on final whole-body lipid content. Enhanced lipid utilization in fish fed with macroalgae-rich diets and consequent lower accumulation of body lipids were observed in previous studies (Dantagnan, Hernández, Borquez, & Mansilla, 2009; Emre, Ergün, Kurtoglu, Güroy, & Güroy, 2013), but could not be confirmed in the present study. These results suggest that fish growth performance and metabolism and possible variations in lipid metabolism may depend not only on the algae species, but also its inclusion level. The relative expression of genes involved in growth and metabolism were evaluated in this study both in liver and muscle. In liver, the expression of *cpt1a* gene, which provides instruction for the enzyme carnitine palmitoyltrasnferase, was downregulated in fish fed $ABVO_{l8+Se1}$ diet. This enzyme is essential for long-chain fatty acid oxidation, a multistep process that metabolizes fats and converts them into energy. This multistep system is part of a fuel sensing gauge, turning off and on fatty acid oxidation depending on the tissue demand for energy (Bremer, 1983; Hoppel & Brady, 1985; Kunau, Dommes, & Schulz, 1995). *Cpt1a* higher expression in fish fed $ABVO_{l8+Se1}$ diet may be a result of a higher use of algae as main lipid source in this diet to replace fish oil. Previous studies suggested that variations on whole-body fatty acid composition due to variable dietary fatty acid profiles could be related to differences on

gene expression, namely *cpt1a* gene (Jin et al., 2017; Morash, Bureau, & McClelland, 2009). Studies on algae supplementation in fish diets revealed potential effects on fatty acids composition in fish. Increasing the amount of algae in diets resulted in increased fatty acid content in fish whole-body and muscle, but was also associated with a variation in the fillet EPA:DHA ratio (M. H. Li et al., 2009; Mourente, Rodriguez, Tocher, & Sargent, 1993). So, it is highly possible that fatty acid composition in fish whole-body, but also in muscle, may have varied between dietary treatments, particularly in those fed on algae biomass supplemented diets. Further analysis on fish whole-body fatty acid composition between dietary treatments could provide a clearer insight on the impact of the transcript level of *cpt1a*. However, the present data does not suggest a significant impact on lipid or energy deposition as nutrient and energy gain remained similar among dietary treatments. The genes involved in growth regulation (*igf1* and *igf2*) were not affected by the diets. Muscle *gpx1a* was the only gene that varied significantly between diets. *Gpx1a* gene expression is related to cell protection activity against oxidative damage as it catalyses the reduction of organic hydroperoxidases and hydrogen peroxidase. Iodine is essential to produce thyroid hormones, it is concentrated as iodide and posteriorly oxidized to produce T4 and later T3, in a process called iodination (Eales & Brown, 1993). These processes cause high oxidative stress, and therefore it is needed a good anti-oxidative defense, using glutathione peroxidases (GPx) and thioredoxin reductase, among others (Beckett & Arthur, 2005; Kohrle, Jakob, Contempre, & Dumont, 2005). The higher iodine content in the $ABFO_{I15+Se1.4}$ diet, as a result of *Laminaria* incorporation, is expected to result in increased biofortification of iodine in fish fillets, and consequently lead to an increased anti-oxidative metabolism. This metabolic response of fish may explain the significantly higher *gpx1a* expression in the muscle of fish fed $ABFO_{I15+Se1.4}$ diet, compared to those fed FMFO diet. Despite relative abundance variations in *cpt1a* and *gpx1a* genes in liver and muscle, respectively, no correlation was observed between fish growth performance and gene expression.

Conclusion

The present results indicate that the concomitant replacement of 33% FM and 20% of FO by a blend of microalgae biomass (*Chlorella, Tetraselmis* and *Schizochytrium)* can be

achieved without any significant impact on growth performance or whole-body composition in large-sized *Sparus aurata*. However, the highest dietary levels of both selenium and iodine resulted in growth impairment. These negative results in fish growth may be caused by an over-supplementation with algae biomass. The higher iodine content in $ABFO_{I15+Se1.4}$ diet, as a result of *Laminaria* incorporation, was associated with an upregulation of the *gpx1a* gene in muscle, probably due to an increased anti-oxidative metabolism. In conclusion, the use of algae biomass as fish meal and fish oil sustainable alternatives may be accomplished but requires a cautious selection of ingredients at adequate level to avoid growth impairment.

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